Adenovirus-Mediated Wild-Type *p53* Gene Transfer in Patients Receiving Chemotherapy for Advanced Non–Small-Cell Lung Cancer: Results of a Multicenter Phase II Study

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Purpose: To study the additional benefit from adenoviral *p53* gene therapy in patients undergoing first-line chemotherapy for advanced non–small-cell lung cancer (NSCLC).

Patients and Methods: Twenty-five patients with nonresectable NSCLC were enrolled in an open-label, multicenter phase II study of three cycles of regimen A, carboplatin (area under the curve, 6; day 1) plus paclitaxel (175 mg/m², day 1), or regimen B, cisplatin (100 mg/m², day 1) plus vinorelbine (25 mg/m², days 1, 8, 15, and 22) in combination with intratumoral injection of 7.5 × 10¹² particles of SCH 58500 (rAd/*p53*, day 1). Responses of individual tumor lesions were assessed after each cycle, and gene transfer was examined in posttreatment tumor biopsies using reverse transcriptase polymerase chain reaction.

Results: There was no difference between the response rate of lesions treated with *p53* gene therapy in addition to chemotherapy (52% objective responses) and lesions treated with chemotherapy alone (48% objective responses). Subgroup analysis according to the chemotherapy regimens revealed evidence for increased mean local tumor regressions in response to additional *p53* gene therapy in patients receiving regimen B, but not in patients receiving regimen A. Th re was no survival difference between the two chemoth r-apy regimens, and the median survival of the cohort was 10.5 months (1-year survival, 44%). Transgene expression was confirmed in tumor samples from 68% of patients, and toxicities attributable to gene therapy were mild to moderate.

Conclusion: Intratumoral adenoviral *p53* gene therapy appears to provide no additional benefit in patients receiving an effective first-line chemotherapy for advanced NSCLC.

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LUNG CANCER IS a leading cause of death from malignancies in the Western World, and non–small-cell lung cancer (NSCLC) accounts for 75% to 80% of all lung cancers.[1] Usually, the only curative treatment available is surgical resection, an option limited to patients diagnosed in early stages of the disease and without significant comorbidity precluding thoracotomy. Patients with nonresectable tumors are treated with palliative chemotherapy or radiotherapy, but the expected survival of patients with stages III B and IV is still less than 20% at 2 years. Thus, new treatments for patients with advanced NSCLC are clearly required.

Structural alterations of the p53 tumor suppressor protein are observed in approximately 50% of tumors of NSCLC patients,[2] and in some, but not all, studies *p53* mutations are associated with an adverse prognosis.[3-5] Mutations of *p53* result in an impaired cellular response to various stresses, including DNA damage, growth factor withdrawal, and oncogenic transformation as well as to genomic instability.[6] Moreover, *p53* loss may also abrogate an effective apoptotic response to chemotherapy or radiation treatment.[7] In preclinical tumor models, *p53* gene transfer as mediated by retroviral or adenoviral expression vectors restored drug and radiation sensitivity or directly induced apoptosis.[8-15] On the basis of its preclinical efficacy, adenovirus-mediated wild-type (wt) *p53* gene transfer[16,17] or wt *p53* gene transfer in combination with cisplatin chemotherapy[18] was studied in phase I trials in patients with advanced NSCLC. These studies established the safety and feasibility of injections of adenoviral *p53* expression vectors into tumor lesions from patients with NSCLC. Moreover, they provided evidence for in vivo transgene expression,[16,17] as well as for efficacy

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in terms of surrogate markers of biologic activity[17,18] and some tumor remissions.[18]

The current multicenter phase II study of local adenovirus-mediated *p53* gene therapy was designed to systematically assess the clinical efficacy of this novel therapeutic approach in patients with incurable NSCLC undergoing an effective first-line chemotherapy.

PATIENTS AND METHODS

Patients

Adult patients with histologically confirmed, chemotherapy-naive, incurable NSCLC with a life expectancy of at least 4 months, a Karnofsky performance score of at least 70%, and absence of any clinical and laboratory evidence for dysfunction of hematopoietic, liver, renal, or coagulation systems were immunohistochemically or molecularly (sequencing or single-strand conformation polymorphism analysis) screened for intratumoral mutation or deletion of the *p53* gene as previously described.[16] Only patients with mutated or deleted *p53* and with two measurable tumor lesions in the same organ that were reasonably comparable in size were eligible for enrollment. An interval of at least 4 weeks between prior surgery and study treatment was mandatory, and all patients were required to provide written informed consent. Pregnant or nursing patients, fertile women not practicing medically accepted contraception, patients with acute adenoviral infections, immunosuppressed patients, HIV-positive patients, patients with uncontrolled serious infections, and patients with a history of acute or chronic respiratory distress were not eligible for enrollment.

Study Design

This was an international, multicenter, open-label, nonrandomized phase II trial. The protocol was approved by the local ethics committees and by the national regulatory offices of each participating center. The study was conducted according to the Declaration of Helsinki (amended version, Hong Kong, 1989), and following the principles of good clinical practice.

Treatment On day 1 of each cycle all patients received a single intratumoral injection of 10 mL of an aqueous solution containing a dose of 7.5×10^{12} particles SCH 58500 (rAd/*p53*), as described previously,[9,16,19] followed or preceded within 2 hours by the initiation of one of the two following chemotherapy regimens: (A) carboplatin (targeted area under the curve of 6, day 1) and paclitaxel (175 mg/m^2 during 3 hours, day 1), or (B) cisplatin (100 mg/m^2, day 1) and vinorelbine (25 mg/m^2, days 1, 8, 15, and 22). Treatment was repeated on day 22 for regimen A, and on day 29 for regimen B, and a maximum of three cycles was planned. Chemotherapy dose modifications were mandatory in case of hematopoietic toxicity. Chemotherapy and premedications were administered as per institutional practices. Intratumoral administration of SCH 58500 was performed either by percutaneous injection under computed tomographic (CT) guidance[20] or by intratumoral injection at bronchoscopy as a single-bolus injection central to the tumor lesion. The vector dose of 7.5×10^{12} particles was chosen based on its established safety as well as its efficacy to induce in vivo transgene expression in patients with advanced NSCLC.[16] After gene therapy, all patients were hospitalized in single rooms in a biosafety environment for at least 24 hours, or until adenovirus shedding was no longer detectable.

Study End Points and Evaluation Procedures The primary objective of the study was to compare the response rates of the SCH 58500-injected lesions to the response rates of the noninjected comparator lesions in patients receiving one of the two chemotherapy regimens. This design was chosen to permit detection of any additional local effect of intratumoral wt *p53* gene transfer by an intrapatient comparison. For response evaluation, CT scans of the chest and tumor measurements of all other extrathoracic tumor lesions were performed at screening, at the last day of each cycle, and every 2 months thereafter, if at least stable disease was achieved after the third cycle. To quantify changes in the size of individual lesions, the largest diameter of each lesion was multiplied with its perpendicular diameter after each treatment cycle, and was compared with the measurements taken at baseline. Remissions were classified according to National Cancer Institute criteria as complete remissions (CR), partial (decrease in the product of the largest and its perpendicular diameter by at least 50%) remissions (PR), stable disease (SD), or progressive (increase in size by at least 25%) disease (PD).

A secondary objective of the study was to assess the safety of the combination of the chemotherapy regimens with intratumoral injection of SCH 58500 for three cycles. Patients were closely monitored for any adverse event after treatment, and after hospital discharge patients were evaluated on a weekly basis by outpatient visits at the study centers. The daily monitoring during the first three posttreatment days included assessment of clinical symptoms, a physical examination, measurements of weight, height, and performance status, recording of adverse events, and frequent assessments of vital signs. Detection of adenovirus shedding, serum chemistry, and urinalysis were performed before and on day 1 of each cycle. Hematology, coagulation profile, ECG, and chest radiograms were assessed before each cycle. Another secondary end point was to assess biologic activity of SCH 58500, as defined by reverse transcription and polymerase chain reaction (RT-PCR) detection of vector-specific wt *p53* RNA sequences in posttreatment tumor biopsies as previously described.[16] For this purpose, on day 2 of each cycle (approximately 24 hours after injection), needle biopsies of the SCH 58500-injected tumor lesions were obtained by the same route by which SCH 58500 had been administered.

Virology Studies Adenovirus shedding was monitored in sputum, stool and rectal swabs, and urine by means of a qualitative enzyme-linked immunosorbent assay (ELISA) before treatment and daily until hospital discharge of the patient, as previously described.[16] Anti-adenovirus type 5 (Ad5) antibodies were detected using a previously described ELISA technique.[16] Neutralizing anti-adenoviral antibodies were assessed by means of the Saos-2 cell proliferation assay. In addition, urine and stool samples were assayed for the presence of infectious adenoviruses by a previously described method[21]: In brief, urine or stool swabs were diluted in growth medium and filter-sterilized. Then, 293 cells growing in logarithmic phase were incubated with the medium and sample mixes for 48 hours, and cells were harvested, stained with a fluorescent-labeled anti-Ad5 antibody, and analyzed by flow cytometry. Infectious titers were expressed as units per milliliter from the number of positive fluorescent cells measured. Any samples scored positive were reincubated with 293 cells for 48 hours. Cells were harvested, DNA was extracted for polymerase chain reaction (PCR) analysis according to standard techniques, and the presence of SCH 58500 sequences was tested using vector-specific primers.[16]

RESULTS

Enrollment and Treatments

Twenty-five patients from eight centers were enrolled in the study between September 24, 1997, and August 7, 1998.

Table 1. Patient Demographics and Localization of Tumor Lesions Receiving SCH 58500 Injections (SCH 58500-treated) and Lesions Serving as Intrapatient Control (comparator lesion)

Patient No.	Age (years)	Sex	Histology	Tumor Stage	SCH 58500-Treated	Comparator Lesion
Carboplatin and paclitaxel treatment						
001	60	male	adenocarcinoma	III B	tumor LLL	tumor tracheobronchial
002	35	female	large cell	IV	tumor RUL	tumor R hilar
004	57	male	squamous	IV	liver met. seg. 5	liver met. seg. 4
006	56	male	squamous	III B	tumor mediastinal	tumor L lung
007	67	male	adenocarcinoma	IV	tumor RUL	tumor R hilar
008	66	male	squamous	III B	ln. met. R supraclavicular	ln. met. tracheobronchial
011	62	male	undifferentiated NSCLC	IV	liver met. seg. 6	liver met. seg. 8
013	51	female	squamous	IV	tumor RUL	tumor LUL
014	83	male	undifferentiated NSCLC	III B	ln. met. supraclavicular	ln. met. aortopulmonal
016	80	female	squamous	IV	tumor RUL	tumor R pleural
017	77	male	undifferentiated NSCLC	III A	tumor L hilar	ln. met. paraaortal
019	44	female	adenocarcinoma	IV	ln. met. L suprascapular	ln. met. L supraclavicular
022	68	male	squamous	III B	ln. met. subcarinal	ln. met. pretracheal
Cisplatin and vinorelbine treatment						
003	50	male	large cell	IV	tumor RML	tumor LUL
005	48	male	adenocarcinoma	III B	tumor LLL	second tumor LLL
009	61	male	squamous	IV	tumor LUL	ln. met. R central
010	56	male	squamous	IV	tumor RUL	tumor lingula L
012	70	male	adenocarcinoma	IV	tumor RUL	ln. met. paratracheal
015	60	male	squamous	IV	chest wall mass	tumor R lung
018	65	female	adenocarcinoma	IV	tumor LLL	tumor RUL
020	50	male	squamous	IV	tumor RML	ln. met. subcarinal
021	47	male	squamous	IV	tumor LUL	ln. met. central
023	57	male	adenocarcinoma	III A	tumor RUL	ln. met. pretracheal
024	56	female	squamous	II B	tumor LUL	second tumor LUL
025	51	male	adenocarcinoma	II B	ln. met. pretracheal	ln. met. mediastinal

Abbreviations: L, left; R, right; LUL, left upper lobe; LLL, left lower lobe; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; ln. met. lymph node metastasis; seg, segment.

Patient characteristics and location of the tumor lesions assessed within the study are summarized in Table 1. A total of 68 treatment cycles were administered in 25 patients, with 20 patients completing the planned three cycles (one patient received two additional cycles, and two patients received one additional cycle each). Three patients received two treatment cycles, and two patients received only one cycle each. In 20 patients, SCH 58500 was administered by percutaneous intratumoral injection under CT guidance, whereas five patients received SCH 58500 by intratumoral injection at bronchoscopy. Thirteen patients were treated with a total of 37 (plus additional four) cycles of chemotherapy regimen A (carboplatin and paclitaxel). Twelve patients received a total of 31 cycles of chemotherapy regimen B (cisplatin and vinorelbine). The two treatment groups were balanced with respect to age and tumor stages (Table 1). Two patients treated with regimen A received intratumoral injections of SCH 58500 into liver metastases. All other patients received the vector injections into thoracic tumors or thoracic lymph node metastases (Table 1). Quantitative tumor responses were assessable for 66 treatment cycles. Tumor measurements were missing for one cycle each in two patients treated with regimen A, and in one patient treated with regimen B.

Clinical Responses

To detect a possible local benefit from additional wt *p53* gene therapy in combination with chemotherapy, the study was designed to analyze for each individual patient the variation of the local responses of two tumor lesions comparable in size and located within the same organ. When the isolated responses of the SCH 58500-injected lesions and the comparator lesions after each patient's last cycle of study therapy were assessed, 52% of the lesions injected with SCH 58500 and 48% of the comparator lesions had an objective response (Table 2). Similarly, no differences were observed when the patients were analyzed separately according to their chemotherapy regimen (54% *v* 46% responses for carboplatin and paclitaxel, and 50% *v* 50% for cisplatin and vinorelbine). In six patients (one treated with regimen A and five treated with regimen B), SCH 58500 was injected into primary lung tumors, and

Table 2. Local Tumor Responses of Lesions*

Patient No.	Cycles	SCH 58500-Treated	Comparator Lesion	Neutral. Ab	RT-PCR
Carboplatin and paclitaxel treatment					
001	3	PR	PR	+	+
002	1	StD	StD	+	+
004	3	PD	PD	+	+
006	3	PR	StD	+	+
007	2	StD	StD	+	–
008	3	PR	PR	+	+
011	3	StD	PR	+	–
013	3	StD	StD	+	+
014	3	CR	PR	+	+
016	2	StD	PD	nd	+
017	3	PR	PR	+	–
019	3	PR	PR	+	+
022	3	PR	StD	+	+
Cisplatin and vinorelbine treatment					
003	3	StD	CR	+	+
005	3	StD	PD	NB	–
009	2	StD	PR	nd	–
010	3	PR	StD	+	–
012	3	StD	PR	+	–
015	3	PR	PR	nd	+
018	2	PR	PR	+	–
020	3	PR	PR	+	+
021	1	StD	StD	nd	+
023	3	CR	StD	NB	+
024	3	StD	StD	+	+
025	3	PR	StD	+	+

Abbreviations: CR, complete response; PR, partial response; StD, stable disease; PD, progressive disease; nd, not determined; NB, no baseline value available; Neutrol. Ab, neutralizing anti-SCH 58500 antibodies; RT-PCR, reverse transcriptase polymerase chain reaction.

*Table represents responses of lesions treated with SCH 58500 injection in addition to systemic chemotherapy and of noninjected lesions comparable in size and located within the same organ (comparator lesion) after the last cycle each patient received within the trial, development of neutralizing anti-SCH 58500 antibodies and RT-PCR detection of transgene expression in posttreatment biopsies.

thoracic lymph node metastases served as comparator lesions (Table 1). No overall difference between the responses of primary tumors (one CR, two PR, and three SD) and lymph node metastases (three PR and three SD) was noted in these six patients (Table 2).

When the areas of the SCH 58500-treated lesions and the comparator lesions were calculated at the end of each treatment cycle, a significant difference (P = .028, Wilcoxon signed rank test) in tumor regressions between the SCH 58500-treated lesions and the comparator lesions of all study patients could only be found after the second cycle (Fig 1A). However, when patients were analyzed separately for each of the two chemotherapy regimens, a different picture emerged: In the 13 patients receiving carboplatin and paclitaxel (regimen A), there was no obvious difference between the mean response of the SCH 58500-treated and the comparator lesions after any of the three cycles (Fig 1B). After the third cycle, a mean regression of approximately 60% was observed in lesions receiving additional gene therapy and in the comparator lesions. In contrast, the mean regression of the comparator lesions of patients who where treated with cisplatin and vinorelbine (regimen B) was only 15%, whereas it amounted to 55% in lesions that were additionally injected with SCH 58500 (Fig 1C). Because of the small sample numbers of the subgroups, no statistical level of significance was calculated.

Regarding the overall outcome of the patients, there was no significant survival difference between the two chemotherapy regimens: Median survival was 10 months in 13 patients treated with carboplatin and paclitaxel, and 13.5 months in 12 patients treated with cisplatin and vinorelbine (P = .328, log-rank test). The median survival of the complete cohort was 10.5 months, and survival at 1 year was 44% (Fig 2).

Detection of p53 *Transgene Expression*

Intratumoral expression of vector-specific wt *p53* RNA could be detected in 12 of 25 patients (48%) undergoing posttreatment biopsies approximately 24 hours after their first SCH 58500 treatment. Another five of 11 patients who had negative RT-PCR results after the first cycle and who underwent additional biopsies had evidence for intratumoral transgene expression after subsequent treatment cycles. Thus, a total of 17 of 25 patients (68%) exhibited transgene expression by positive RT-PCR for vector-specific wt *p53* sequences (Table 2).

Toxicities

Toxicities attributable to treatment with SCH 58500 generally were mild to moderate. Most frequently fever, influenza-like symptoms, nausea or anorexia, and fatigue were observed. Injection-site complications occurred during only five of 53 cycles in which SCH 58500 was administered by CT-guided percutaneous injection (one with World Health Organization [WHO] grade 2 pain and four with WHO grade 1 reactions). All toxicities of WHO grade 2 or higher, which occurred during at least two of 68 cycles, are summarized in Table 3.

Unscheduled hospitalizations occurred because of decreased performance status (two patients), renal failure (one patient after an additional fourth cycle), febrile neutropenia (one patient), bacteriemia in the absence of fever (one patient), and hypercalcemia (one patient), none of which was considered as probably related to SCH 58500 treatment. In five patients the study had to be discontinued prematurely: three patients had disease progression after the second cycle, one patient experi-

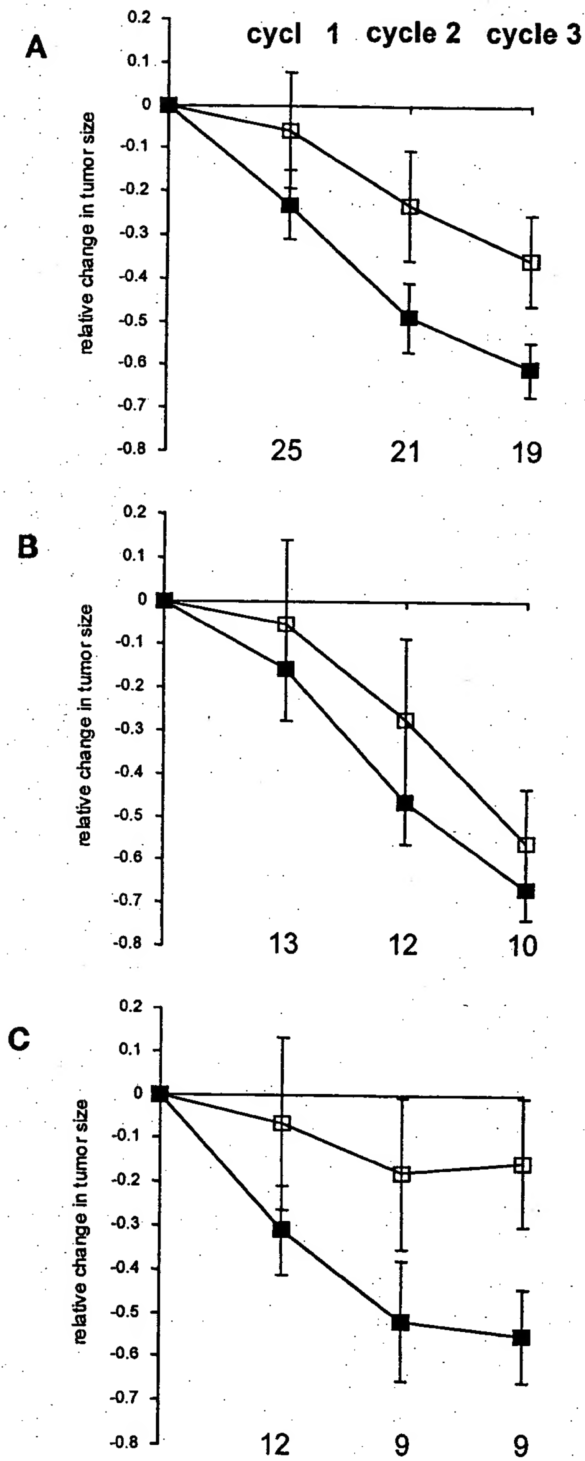

Fig 1. Quantitative tumor responses after wt *p53* gene therapy plus chemotherapy. Mean relative areas changes ± SE of SCH 58500-injected tumors (closed squares) and comparator lesions (open squares) of the whole study cohort (A), patients treated with carboplatin and paclitaxel (B), and patients treated with cisplatin and vinorelbine (C). (Numbers at bottom of each graph indicate the number of assessable patients.)

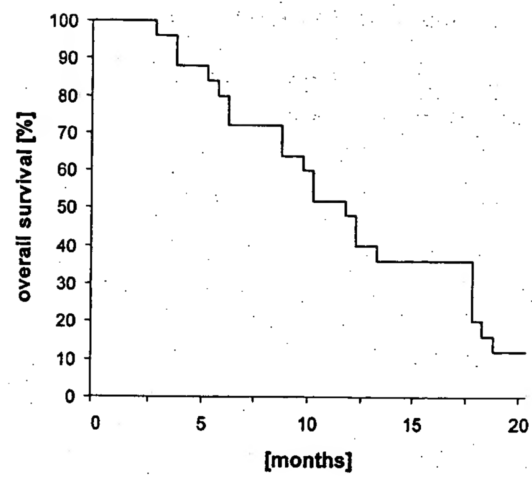

Fig 2. Actuarial survival of 25 patients treated with intratumoral injections of SCH 58500 in combination with chemotherapy (Kaplan-Meier analysis).

enced asthenia and leukocytopenia after the first cycle leading to discontinuation, and one patient had a remission of the injected lesion after the first cycle, which made it technically impossible to reinject SCH 58500 during subsequent cycles.

Changes in laboratory variables were mainly restricted to blood counts, which was not unexpected in the light of the known toxicities of the two chemotherapy regimens ap-

Table 3. Treatment-Related Toxicities of at Least WHO Grade 2 Observed in at Least Two of 68 Cycles of SCH 58500-Injection in 25 Patients

	WHO Grade		
Toxicity	2	3	4
Anorexia	9	—	—
Arthralgia	2	—	—
Asthenia	5	1	—
Constipation	6	—	—
Cough	4	1	—
Dehydration	3	—	—
Diarrhea	6	—	—
Dyspnea	3	2	—
Emesis	4	2	—
Fatigue	8	3	—
Fever	18	1	—
Headache	1	1	—
Hypertension	3	—	—
Influenza-like symptoms	2	1	—
Myalgia	2	—	—
Nausea	14	—	—
Pain	12	1	—
Sweating	2	—	—

NOTE. The numbers indicate the maximal severity of the respective adverse event per subject.

plied. A WHO grade of greater than 3 leuko- or neutropenia occurred in six of 13 patients (46%) treated with regimen A (carboplatin and paclitaxel), and in 11 of 12 patients (92%) treated with regimen B (cisplatin and vinorelbine). A WHO grade of greater than 3 thrombocytopenia was observed in two of 13 patients (15%) receiving regimen A, and one of 12 patients (8%) receiving regimen B. In addition, a WHO grade 2 increase of transaminases was observed in three patients, and a WHO grade 2 increase of alkaline phosphatase was observed in one patient treated with regimen A. Four patients treated with regimen B experienced a WHO grade 2 increase in transaminases. One patient treated with regimen A had a WHO grade 3 renal insufficiency after a fourth treatment cycle, which he received outside the protocol. In another patient, a reversible WHO grade 2 hyponatremia was observed.

Virology Studies

In two of 25 patients adenovirus shedding in the urine was detected by means of the on-site ELISA. Excretion of infectious adenoviral particles (4.35×10^4 U/mL) was detected in a stool sample from one patient (patient no. 011), obtained 24 hours after SCH 58500 injection during the second cycle. However, no vector-specific DNA sequences were found in this sample using PCR. No adenoviruses were detected in a urine sample taken at the same time, or in stool and urine samples obtained 48 hours after treatment. This patient was one of the two patients receiving SCH 58500 injections into liver metastases (Table 1).

Positive anti-Ad5 antibody titers were detected in all 19 patients for whom pretreatment serum samples were available. After the first injection of SCH 58500, the anti-Ad5 serum antibody titers increased by a mean of 3.5-fold at day 7, and by a mean of 35-fold at day 14 after injection (Fig 3A). During subsequent treatment cycles anti-Ad5 antibody titers remained at these increased levels (data not shown). In none of the 19 patients in whom pretreatment serum analysis could be performed were neutralizing anti-SCH 58500 serum antibodies detectable at baseline. After SCH 58500 injection, neutralizing antibodies developed in all of these 19 patients. Neutralizing antibodies were detectable as early as day 7 of the first cycle (Fig 3B) and remained detectable in all patients assessed throughout the study (data not shown).

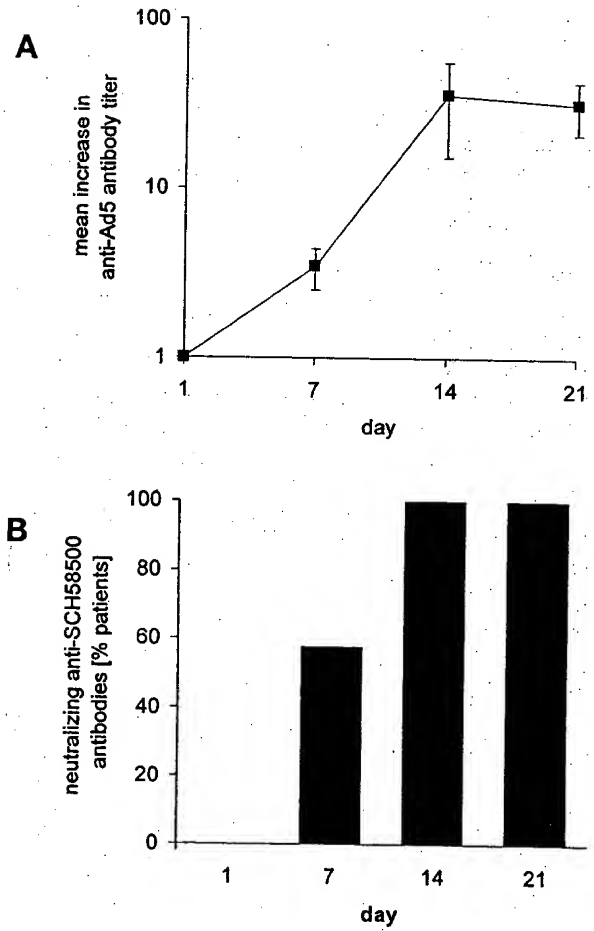

Fig 3. Development of anti-adenoviral serum antibodies and neutralizing anti-SCH 58500 serum antibodies after the first treatment with SCH 58500 in 19 assessable patients. (A) Mean increase (± SE) above baseline of anti-adenoviral antibodies. (B) Fraction of patients with detectable neutralizing anti-SCH 58500 antibodies.

DISCUSSION

Transfer of the tumor suppressor gene *p53* was shown to induce permanent cell cycle arrest or apoptotic cell death in a large number of cancer cell lines, as well as in several preclinical animal models of cancer.[10,13,22,23] An apoptotic response to *p53* activation appears to be limited to transformed cells, whereas nontransformed cells mainly undergo cell cycle arrest after *p53* activation.[24,25] Encouraging preclinical results led to a number of early clinical studies exploring vector-mediated *p53* gene transfer in advanced cancer patients. The earlier studies were performed in patients with incurable NSCLC,[16,26] or head and neck squamous cell cancer.[27] A pilot study[26] in which retroviral *p53* expression vectors were directly injected into small endobronchial lesions received widespread publicity and gave increase to high hopes for the efficacy of *p53* gene therapy of cancer. As the vector technology applied in these trials did not allow selective tumor targeting, and systemic administration of these vectors was precluded because of their high immunogenicity and the high prevalence of cross-reacting antibodies, direct approaches of tumor target-

ing were applied. The safety and feasibility of intratumoral injection of adenoviral wt *p53* expression vectors was established in NSCLC patients[16,17] and in patients with head and neck squamous cell cancer.[27] Furthermore, evidence for transgene expression, and possibly induction of apoptosis, was documented by these trials.[16,17,27] Additional trials evaluated infusion of adenoviral vectors into body cavities[28] or tumor perfusion through intra-arterial injection of vector solutions[29] as other targeting techniques.

Intratumoral vector injection can only be applied to a limited number of lesions per patient, and currently there is no clinical evidence to support systemic antitumoral effects from such a treatment. Thus, it is difficult to assess the clinical activity of local *p53* gene transfer in patients with advanced NSCLC after standard oncologic practice. Furthermore, response data from controlled phase I studies of adenoviral wt *p53* gene transfer[16,17] did not reproduce the impressive local effects, which were initially reported using a less-effective retroviral expression system.[26] To overcome those limitations, the novel design of the present study was chosen. By comparing the isolated responses of a tumor lesion treated with adenoviral wt *p53* gene transfer with a comparable lesion not receiving gene therapy in patients undergoing first-line chemotherapy, we hoped to obtain a meaningful assessment of additional effects from local wt *p53* gene therapy in NSCLC. These restrictive inclusion criteria naturally raise the risk of selection bias in our study. However, we believe that our observations are valid, inasmuch as responses were not compared with historical controls but within each individual patient, thus minimizing the influence of biologic differences among patients as well as their tumors on the efficacy outcome.

In doing so, we found no convincing evidence for an additional local benefit from adenoviral wt *p53* gene transfer in NSCLC patients undergoing first-line chemotherapy. This result also held true, if quantitative regressions of mean tumor sizes were compared instead of the overall responses of each lesion (Fig 1A). One possible explanation for this observation could be the relatively high efficacy of the chemotherapy administered, as well as the small cohort size of our study. Response rates of 50% certainly are in the upper range of those reported in phase II studies of chemotherapy alone.[30-32] However, this is not surprising given the relatively good performance status of the patients included in this study.

Second, the lack of a detectable clinical benefit from SCH 58500 injection could hypothetically result from biologic inactivity of the study medication in our study patients. This, however, appears highly unlikely, as this vector formulation has extensively demonstrated biologic activity in various preclinical tumor models comparable to the clinical setting of this trial.[11,33,34] Moreover, transgene expression as determined by RT-PCR analysis in 24-hour posttreatment tumor biopsies was confirmed in 68% of the study patients (Table 2). As this trial was mainly designed to address the clinical efficacy end point, no systematic studies of additional surrogate markers for treatment-associated induction of cell cycle arrest or apoptosis were performed. However, using quantitative RT-PCR methodology, upregulation of mRNA of the *p53*-target gene *p21/WAF1* after SCH 58500 administration was observed in a subgroup of this study cohort.[35]

Another reason for the lack of an additional benefit from *p53* gene therapy might be insufficient spreading of the replication-defective adenoviral vectors within the tumors after central intralesional injection. As surgery was not indicated in the patients enrolled in the present trial, this factor could not be assessed in this study. Addressing this important question should be considered in future studies of adenoviral cancer gene therapy performed in early stage patients scheduled for surgery. Recently, the problem of ineffective vector spreading has been attempted to be overcome by administration of replication-competent adenoviruses,[36,37] and encouraging clinical results were reported.[38,39] However, using replication-competent viruses, even on the background of preferential replication in cells with mutations in the ARF-p53-Rb pathway, might pose additional safety concerns.

Toxicities observed in this study mainly resulted from the concomitant chemotherapy, and they were within the range of those described from large phase II or phase III trials of the same agents alone in advanced NSCLC patients.[30-32,40,41] Toxicities attributed to the gene therapy itself were mild to moderate, and confirmed results from phase I trials of adenoviral *p53* vector injection alone.[16,17]

Vector-specific wt *p53* RNA expression could be detected in posttreatment biopsies from a high number of patients (68%). This demonstrates a high quality of study conduct, which was important because the majority of centers enrolled only three or fewer patients in this multicenter trial. Although excretion of infectious adenoviral particles could be detected during only one of 68 treatment cycles, high titers of adenovirus-reacting antibodies and neutralizing anti-adenoviral antibodies became detectable in all assessable patients after gene therapy. These antibodies developed even though all patients received a highly myelotoxic chemotherapy and most patients were premedicated with dexamethasone. This supports the known immunogenicity of adenoviral vectors in humans. Median and 1-year survival, as well as the toxicities observed in this trial, compared favorably with those from studies of similar chemotherapy regimens,[30-32,40,41] establishing the safety of

multiple intratumoral injections of SCH 58500 in combination with chemotherapy in patients with advanced NSCLC.

Although our study patients were not randomly assigned to chemotherapy regimen A or B, retrospective subgroup analysis still provided some potentially interesting information. If the comparison of the chemotherapy response was limited to the comparator lesions, which did not receive additional gene therapy, higher mean tumor regressions were observed after treatment with carboplatin plus paclitaxel than after cisplatin plus vinorelbine (Fig 1, B and C). In the former group no additional benefit could be found in lesions treated with *p53* gene therapy. In contrast, the latter group exhibited a difference in the local effect from gene therapy combined with chemotherapy, which was most pronounced after the second and third cycles. This has to be interpreted with caution, given the small sample size and the retrospective nature of the analysis. However, this might suggest some additional local effects from adenovirus-mediated wt *p53* gene transfer on the background of a less than optimal response to systemic chemotherapy. In contrast, local *p53* gene therapy does not appear to provide an additional benefit, if the overall response to systemic chemotherapy is optimal.

Relevant benefits from adenovirus-mediated wt *p53* gene therapy to the large population of patients with advanced, inoperable NSCLC in terms of overall response rate and survival, however, can only be assessed in randomized phase III trials. Whether such a study is warranted on the basis of the results obtained in this first phase II study, using current vector technology, which is limited to local application, remains open.

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